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Experimental Methods for Evaluating the Health Risks Associated with Organic Chemicals in Drinking Water

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16. ABSTRACT

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Research conducted by our laboratory indicates that there is little evidence to suggest that chloroform acts through interaction with DNA or as a tumor initiator. This conclusion is based upon the absence of activity in the Ames assay, little or no evidence of direct chemical interaction with DNA *in vivo*, and inability to show clear evidence of tumor initiating activity in the rat liver or mouse skin. Chloroform does, however, produce clear evidence of liver damage in susceptible species at doses associated with increased tumor incidence. On the other hand, chloroacetonitrile and dibromoacetonitrile, compounds also found in drinking water, clearly act as tumor initiators in the mouse skin. Although the potencies of these chemicals as whole carcinogens have yet to be established, it is clear that assessment of the risks they pose will have to be made on a different basis than for chloroform.

Experimental systems designed to distinguish between tumor initiating and promoting activity are not presently accepted as a basis for making quantitative estimates of risk. However, data from such systems should be considered when risk assessment models for low dose extrapolation of data obtained from the more widely accepted lifetime carcinogenesis bioassay or from epidemiological data are being selected. Intelligent use of these experimental approaches provide a more accurate basis for assessing the relative hazards of carcinogens than the conservative approach of treating all chemicals that increase tumor incidence in a standardized way.

KEYWORDS: Carcinogens, initiation, promotion, haloacetonitriles, chloroform.

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Experimental Methods for Evaluating the Health Risks Associated with Organic Chemicals in Drinking Water

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Estimation of health hazards associated with the widespread distribution of small amounts of chemical carcinogens in drinking water presents a complex problem. However, it is becoming clear that risks attributed to chemical treatments that increase tumor incidence in experimental animals may be profoundly affected by the type of mechanism responsible for tumor induction. Differentiation of mechanisms involved in carcinogenesis can be made between those chemicals acting through interactions with cellular DNA and those acting through mechanisms not involving the genome. Chemicals commonly found in drinking water fall into both categories.

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INTRODUCTION

Identification of a wide variety of organic chemicals in surface and ground waters has brought considerable attention to the problem of estimating risks these chemicals pose to human health. Considerable uncertainty is involved in making such estimates because in most instances the extrapolations involved go beyond the realm of actual scientific data. First, it is generally necessary to assume that humans respond in a manner that is not only qualitatively but quantitatively similar to the test animal from which evidence of adverse effect of a chemical is obtained. Second, it must be assumed that the effects seen at high doses can be extrapolated to the usually much smaller doses that are encountered in the general environment. This latter assumption is necessary even where human data is available since the sensitivity of epidemiological studies are quite low except where a very specific disease state can be attributed to a chemical.

Chemicals which produce cancer pose some of the most difficult problems in extrapolation. The somatic mutation theory of chemical carcinogenesis has placed considerable emphasis on genotoxic mechanisms in initiating carcinogenesis. Chemicals which act through such a mechanism would be presumed to possess a linear relationship between dose and response at low response rates.[1] Making the assumption that all chemicals that give rise to tumors in life time bioassays act through a genotoxic mechanism leads to the conclusion that human exposure must be kept at very low concentrations to maintain risk at an acceptably low level.

In recent years it has been shown that many commonly used bulk chemicals produce tumors at high doses in experimental animals. The view has been expressed that some of these chemicals produce cancer through a non-genotoxic mechanism. These arguments are based on the observation that such chemicals produce overt toxicity in the target tissue which results in a regenerative hyperplasia. The present paper attempts to indicate critical areas of basic toxicological research that are necessary to resolve the scientific issues in this area.

Initiation vs. promotion of tumors

A simplified view of processes involved in chemical carcinogenesis is depicted in Figure 1. According to the somatic mutation theory of carcinogenesis, tumors arise from genetically altered cells which proliferate to form tumors.[2] Chemicals which are capable of initiating this process, particularly through a direct interaction with DNA, are termed initiators. The lesion produced is such that DNA is replicated containing an error

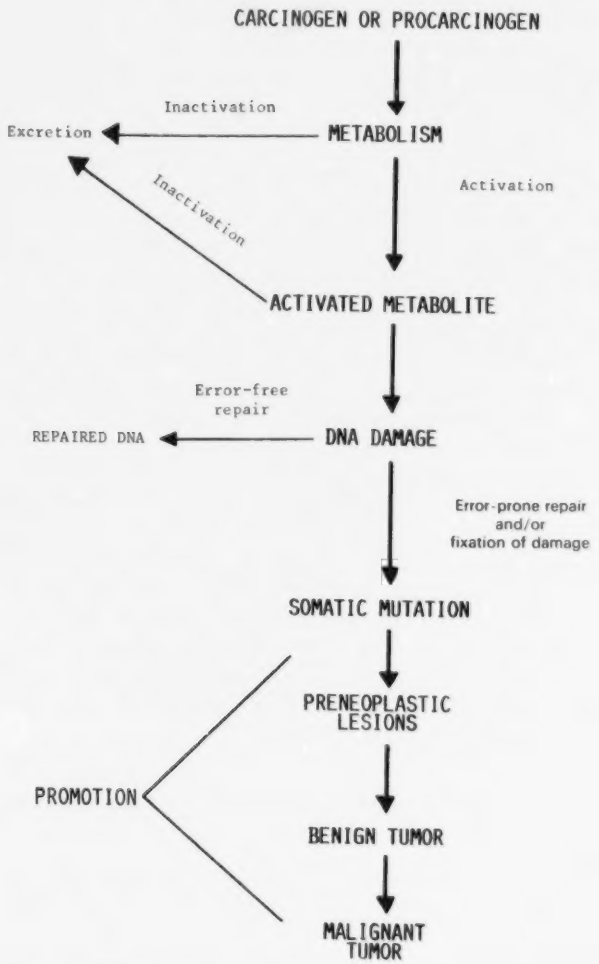

FIGURE 1 Simplified view of biological events involved in chemical carcinogenesis.

leading to a permanent, or fixed alteration in the genotype of the cell. The form of the chemical which reacts with DNA is usually electrophilic.[3]

A substantial fraction of chemical carcinogens are not electrophilic but are metabolized to electrophilic agents within the body.[3] Often the metabolism of xenobiotic chemicals involves multiple pathways. Significant alterations in the qualitative (change in predominant pathway)

or quantitative (rate) patterns of metabolism will govern the concentration of the "active" intermediate at the target site. Not only are there pathways for activation of chemical carcinogens, but there are also detoxification pathways present. Obviously, selecting alterations in steps involved in activation or detoxification steps will significantly affect the biological response.

Once damage is done to DNA, there are mechanisms which are capable of repairing the damage. Such repair may be necessary for the cell to divide. Repair can be effected by either error-free or error-prone mechanisms.[2] If DNA is replicated without repair or after repair with an error-prone system, the genetic lesion is "fixed" and may be passed to successive generations of the same cell. Again, if secondary factors affect the repair process the tumorigenic response can be considerably altered.

Given the existence of a cell or group of cells that are genetically altered to possess those properties (whatever they might be) that allow uncontrolled cellular division and growth, another process can intervene that may in large part determine the impact of tumor initiation on life expectancy. This process is termed tumor promotion. Promoters are chemicals which increase the tumor yield in a given time interval or decrease the time for a tumor to reach some easily recognized size. Such an effect can be mediated through stimulation of cell division. If this stimulation is partially or completely specific to initiated cells even more rapid development of tumors would be anticipated. Although the actual mechanisms involved are poorly understood, hyperplasia is closely associated with tumor promotion.[2] However, all chemicals which produce hyperplasia do not act as tumor promoters.[5,6]

Carcinogenic chemicals associated with chemicals identified in drinking water contamination

Table I lists a number of chemicals which occur in drinking waters that have been shown to produce tumors in at least one species following chronic exposure. Such evidence has been traditionally accepted as the means of defining the carcinogenic properties of chemicals. However, the usefulness of such data as the sole means of making risk assessments at the low exposure levels encountered in the environment should perhaps be questioned.

The importance of calling attention to the multistep nature of chemical carcinogenesis is to point out that chemicals can increase the incidence of tumors by a variety of mechanisms. Involvement of different mechanisms naturally leads to different assumptions concerning the nature of the dose-response relationship at low response rates. In the case of genotoxic

TABLE I

Carcinogenicity of selected chemicals found in drinking water

Chemical	Species	Site (S)	Reference
Arsenic	Human	Skin, lung	35
Benzene	Human	Leukemia	36
Vinyl chloride	Human	Liver, brain, lung	37
	Rat	Liver	37
	Mouse	Lung, liver, mammary gland	37
1,1-Dichloroethylene	Mouse	Kidney	38
Trichloroethylene	Mouse	Liver	39
Tetrachloroethylene	Mouse	Liver	40
1,2-Dichloroethane	Rat	Liver, spleen, stomach	41
1,1,2-Trichloroethane	Mouse	Liver, adrenal	42
1,1,2,2-Tetrachloroethane	Mouse	Liver	43
1,2-Dibromoethane	Rat	Stomach, liver, adrenal	44
	Mouse	Lung, stomach	
1,2-Dibromo-3-chloropropane	Rat	Stomach, mammary gland	45
	Mouse	Stomach	
Chloroform	Rat	Kidney, thyroid	23
	Mouse	Liver	
Carbon tetrachloride	Mouse	Liver	39
2,4,6-Trichlorophenol	Rat	Leukemia	46
	Mouse	Liver	

chemicals it is assumed that certain factors, such as metabolism, are linear at low response rates. Once an altered genome is produced by such a chemical that is capable of being replicated, an irreversible process has been initiated. Since any molecule of the genotoxic agent or its metabolite has a finite probability of interacting to produce the appropriate change in DNA, a linear relationship is predicted on biological grounds.

A biological argument cannot be made for a linear dose response for chemicals that increase the yield of tumors through cocarcinogenic or tumor promoting mechanisms. Although conclusive evidence is also lacking for the converse argument, a substantial body of data would suggest that tumor promotion requires treatments that produce definite signs of hyperplasia and this response must be maintained over time.[7] To result in tumors these effects depend upon the presence of genetically altered cells which arise spontaneously or as a result of other factors. The need for consistent exposure also implies that damage is not cumulative and is reversible, at least to a point (i.e., the tumor has reached some critical size). With the current lack of information concerning specific biochemical mechanism(s) for tumor promoters and cocarcinogens it is difficult to predict the nature of the dose-response at low response rates. It

can only be stated that there is no substantive reason for assuming the linear relationship predicted for tumor initiators.

It is possible to conceive of chemicals which act only as tumor initiators or as tumor promoters. In such an extreme world, initiation and promotion can both be considered relatively benign biological processes since a combination of these processes would be necessary for a tumor to develop. In reality, recognized chemical carcinogens tend to possess both properties and are referred to as complete carcinogens. Consequently, it becomes important to establish which mechanism predominates in a given situation.

Arsenic and benzene are two chemicals which are accepted as human carcinogens. The fact that these chemicals have been uniformly negative in animal experiments could be due to one of two reasons: 1) the experimental animals utilized are relatively resistent to the carcinogenic properties of these chemicals; *or* 2) the carcinogenic effects of these chemicals are due to an interaction between these chemicals and secondary factors present in the human environment and excluded from the animal experiments. Epidemiological studies cannot easily distinguish between these two alternatives. There is only the weakest evidence that either arsenic or benzene possess genotoxic properties (Table II). This tends to support the alternative that secondary factors might be involved in the human response to these agents. Determining whether these two chemicals act through non-genotoxic mechanisms is critical not only to the assessment of the hazards they pose to human health, but to the whole process of assessing human health risks using animal data.

Vinyl chloride, on the other hand, is a chemical whose carcinogenicity has been easily confirmed in experimental animals. The chemical produces tumors in multiple species and multiple target organs (Table I) and clearly possesses genotoxic activity in a number of test systems (Table II). This is despite the fact that it is a fairly weak carcinogen in terms of the level of exposure required to produce cancer.

More highly substituted ethylenes display a somewhat different pattern. Trichloroethylene and tetrachloroethylene were demonstrated to only produce liver tumors in the B6C3F1 mouse (Table I) despite the fact that both chemicals were tested in rats as well. Trichloroethylene displays weakly mutagenic effects (Table II) whereas tetrachloroethylene is essentially without activity.[8] The mutagenic effects of trichloroethylene in *Salmonella typhimurium* have been largely attributed to impurities present in the technical product.[7] Trichloroethylene has been specifically tested for tumor initiating activity in the rat liver and found to be inactive.[10] Both trichloroethylene and tetrachloroethylene are noted for their ability to produce liver damage.[11,12] Additionally, covalent interaction of

TABLE II

Genotoxicity of chemicals identified in drinking water that possess genotoxic properties

Chemical	Evidence of genotoxicity	Reference
Halomethanes		
Chloroform	DNAR, *S. cerevisiae*	47
Dibromochloromethane	GM, *S. typ.*	24
Bromochioromethane	GM, *S. typ.*	24
Bromodichloromethane	GM, *S. typ.*	24
Bromoform	GM, *S. typ.*	24
Halo-alkanes and -alkenes		
1,2-Dichloroethane	GM, *S. typ.*	48, 49
	GM, *D. melanogaster*	50
1,1,2,2-Tetrachloroethane	GM, *S. typ.*	49
	DNAR, *E. coli*	51
Vinyl chloride	GM, *S. typ.*	52, 53
	GM, *Schizosaccharomyces pombe*	54
	GM, *Hamster, in vitro*	55
	SCE, *Human*	56
	CA, *Hamster*	57
	CA, *Human*	57
Hexachloro-1,3-butadiene	GM, *S. typ.*	26
1,1-Dichloroethylene	GM, *S. typ.*	58
	GM, *E. Coli.*	8
Trichloroethylene	GM, *S. typ.*	24
	GM, *S. cerevisiae*	59
	OT, *Fisher rat embryo*	60
Chlorinated Benzenes and Phenols		
1,3-Dichlorobenzene	GM, *Aspergillus nidulans*	61
1,4-Dichlorobenzene	GM, *Aspergillus nidulans*	61
Benzylchloride	GM, *S. typ.*	62, 63
2,4-Dichlorophenol	CA, *Vicia Faba*	64
Nitrogen Containing		
2-Choroaniline	DNA Damage *E. coli*	62
	GM, *Aspergillus nidulans*	61
3,4-Dichloroaniline	GM, *Aspergillus nidulans*	61
Ethyl carbamate	GM, *S. typ.*	65
	CA, *Hamster*	66
	MM, *Test in vivo*	67
	TI, *Mouse Skin*	68
4-Nitrobenzoic acid	GM, *S. typ.*	69
Caffeine	DNA damage, *E. coli*	70
Dichloroacetonitrile	GM, *S. typ.*	23, 71
Chloroacetonitrile	TI, *Mouse Skin*	Present paper
Dibromoacetonitrile	TI, *Mouse Skin*	Present paper
Alcohols		
1-Phenylethanol	GM, *Saccharomyces sake'*	72
2,2,2-Trichloroethanol	GM, *Aspergillus nidulans*	73

TABLE II (cont.)

Chemical	Evidence of genotoxicity	Reference
Aromatic Hydrocarbons		
Styrene	GM, *S. typ.*	74
	GM, *D. melanogastor*	75
	CA, *Hamster, in vitro*	76
	CA, *Human*	77
Pyrene	GM, *S. typ.*	78
Phenanthrene	SCE, *Hamster, in vivo*	79
2,3,6-Trimethylnaphthalene	GM, *S. typ.*	78
Anethole	GM, *S. typ.*	80
Pesticides and Derivatives		
P,P'-DDE	GM, *Hamster, in vitro*	81
	CA, *Hamster, in vitro*	81, 82, 83
2,4-Dichlorophenoxyacetic acid	CA, *Vicia faba*	64
	CA, *Human, in vitro*	84
	CA, *Hamster, in vitro*	85
	CA, *Mouse, in vivo*	84
2,4,5-Trichlorophenoxy-acetic acid	GM, *D. melanogaster*	86
	CA, *Vicia faba*	64
	CA, *Gerbil*	87
Miscellaneous		
Dimethyl sulfate	GM, *S. typ.*	86
	GM, *G. Melanogaster*	89
	GM, *Hamster, in vitro*	90
	GM, *Human, in vitro*	91
Furaldehyde	GM, *S. typ.*	92
4-methylquinoline	GM, *S. typ.*	78
Sorbic acid	CA, *Hamster, in vitro*	93
Triethylphosphate	GM, *S. typ.*	94
	GM, *P. aeriv.*	94
	GM, *D. melanogaster*	94
Bis(2-ethylhexyl)adipate	CA, *Mouse*	95
Bis(2-chloroethyl)ether	GM, *S. typ.*	24
Arsenic	OT, *Hamster, in vitro*	96

DNAR = DNA Repair
GM = Gene mutation
CA = Chromosomal abnormalities
OT = Oncogenic transformation
MM = Mouse micronucleus
SCE = Sister chromatid exchange
TI = Tumor initiator

Note: Information partially assembled under Contract with the AWARE Corp., Dr. D. Goodman.

trichloroethylene with DNA has been shown to be very low.[11] Therefore, the likelihood of a tumor promoting mechanism being predominantly responsible for their carcinogenic effects in the mouse liver is still great despite indications of weak genotoxic effects. Similar possibilities exist for other chemicals in Table II which produce tumors only at doses which produce a hyperplastic responses in the target organs.

It is clear from these considerations that the lifetime bioassays in experimental animals cannot distinguish between tumor initiators or promoters any better than epidemiological studies. The critical questions are: 1) how can initiating and promoting activity be differentiated experimentally; and 2) in the case of a chemical that possesses both types of activity what proportion of the result can be attributed to each mechanism?

The mouse skin has been the classical system in which such activities have been separated and demonstrated.[13] More recently the 2-stage carcinogenesis has been demonstrated in other organs most notably the liver,[14,15] bladder,[16] mammary gland,[17] colon[18] and lung.[19] These studies indicate that multi-stage carcinogenesis is a general phenomena and demonstrate that experimental systems are available for distinguishing between tumor initiation and promotion.

Treatment	Result
I	No tumors
PPPPPPPPPP	No tumors
I PPPPPPPPPP	Tumors
I PPPPPPPPPP	Tumors
I $10 \times$ P	No tumors
I P P P P P P P P P P	Reduced response

FIGURE 2 General experimental design for initiation and promotion experiments. I = tumor initiator; P = tumor promoter.

Figure 2 illustrates the general form of initiation/promotion experiments. The definition of tumor promotion is critically dependent upon the order of treatments.[20] To be effective, tumor promoters must follow treatment with an initiator. Since the effects of the initiator are irreversible, the exposure of the promoter can be delayed and given rise to the same result. On the other hand the promoter must be administered at some minimum frequency to be effective. It is not effective if administered at the same total dose as a single treatment. The response can also be greatly attenuated if the time between treatments becomes great enough that maintenance of the hyperplastic state is reduced.

Chloroform is a chemical of great importance as a contaminant of drinking water because it is produced as a by-product of chlorination.[21,22] The National Cancer Institute carcinogenesis bioassay[23] demonstrated that chloroform administered by gavage in corn oil is capable of inducing tumors in both rats and mice. As noted in Table II. chloroform has been shown to initiate DNA repair in yeast. However, it has proven negative when tested as a mutagen in *S. typ.*[24,25,26] and in hamster cells *in vitro.*[27] Additionally, binding of ^{14}C-chloroform to DNA in mouse and rat liver and kidney has been shown to be extremely low *in vivo.*[28,29] Therefore, evidence that chloroform acts through a genotoxic mechanism is weak and indirect.

Through studies being conducted at Stanford Research Institute our Laboratory is attempting a finer examination of the dose-response characteristics of chloroform carcinogenesis. In these studies chloroform is being administered in the drinking water to avoid the complications of repeated gavage and the high fat intake resulting from the use of corn oil as the vehicle. Even under these conditions it is notable that there is evidence of increased liver fat by both chemical analyses and histopathological examination in the B6C3F1 mouse (Table III). On the

TABLE III

Liver fat content and pathology in B6C3-F1 mice treated with chloroform in drinking water

Chloroform mg/l	Percent liver fat[a]		Number of animals with centrilobular fatty change[a]			
	90 days[d]	180 days	30 days	60 days	90 days	Total
0	3.33	5.82	0	0	0	0
200	3.45	7.93[b]	0	0	0	0
400	3.89[c]	6.77	3	0	0	0
600	—	—	0	0	0	0
900	4.51[c]	7.11[b]	2	0	0	2
1800	6.36[c]	10.40[c]	5	0	4	9
2700	—	—	6	5	2	13

[a]Average value for 10 animals at each dose and time.
[b]Statistically significant from control at P 0.05 by ANOVA and t-test.
[c]Statistically significant from control at P 0.01 by ANOVA and t-test.
[d]Duration of exposure to chloroform at the indicated concentrations.

other hand, little or no evidence of such liver toxicity was observed in the Osborne–Mendel rat (Table IV). The results of the lifetime study at SRI have yet to be analyzed. It is relatively clear though that sensitivity to chloroform-induced tumors in the NCI bioassay[23] does parallel the species sensitivity to chloroform-induced hepatotoxicity (The 900–

TABLE IV
Liver fat content in osborne-mendel rats exposed

Chloroform mg/l	Percent liver fat[a] 90 days	180 days
0	3.32	4.49
200	3.31	4.50
400	3.20	4.59
900	3.58	4.77
1800	3.46	5.13[b]

[a]Average value for 10 animals at each dose and time.
[b]Significant at P 0.05 by NOVA and t-test.

2700 mg/l doses closely approximate the daily doses employed in the NCI study). This result is consistent with the observations of Eschenbrenner[30] who showed a parallel between liver damaging doses of chloroform and development of hepatomas.

Following up on this background of information, Pereira and coworkers[29] have attempted to determine whether chloroform can be shown to act as a tumor initiator or promoter in the rat liver. The endpoint for these experiments involves the identification of focal areas of liver parenchyma that stain for an enzyme, gamma glutamyl transpeptidase, which is not normally found in adult rat liver parenchyma. These focal changes in enzyme activity are consistent with histochemical changes observed in hepatocellular carcinomas and are felt to be indicative of preneoplastic change in the liver.[31]

The essential results have been abstracted from Periera *et al.*[29] in Table V (Note: Protocols for initiation assay and promotional assay of chloroform varied somewhat. See original ms. for details). Tested as an initiator, it is reasonably clear that chloroform was without significant effect. This is in sharp contrast to the results obtained with diethylnitrosamine, an established tumor initiator in the rat liver.

In the test for promoting activity chloroform did induce more than a doubling of the number of foci/cm^2. However, this result was not statistically significant. The positive control, barbital, produced a significant response, but was considerably weaker than has been generally observed. This latter finding suggests that the sensitivity of the assay was somewhat below normal in these experiments and that they need to be repeated.

Although these results are not conclusive, they do indicate that chloroform possesses few of the properties normally associated with an

TABLE V

Initiating and promoting activity of chloroform in the rat liver

Initiator	Promoter	GGTase-foci foci/cm^2
	Initiation assay	
Tricaprylin (Control)	Phenobarbital	1.07 ± 0.24
Chloroform	Phenobarbital	1.08 ± 0.28
Diethylnitrosamine	Phenobarbital	15.8 ± 1.9
	Promotion assay	
Diethylnitrosamine	Tricaprylin (Control)	0.90 ± 0.31
Diethylnitrosamine	Chloroform	2.22 ± 0.63
Diethylnitrosamine	Barbital	3.02 ± 1.10

[a]Abstracted from Pereira *et al.*, 1982.

initiator and has little or no such activity in the rat liver. There is, on the other hand, a suggestion that chloroform could act as a tumor promoter in the rat liver, although the data obtained to date is equivocal. Obviously, this question needs further research *and* should be extended to determining the mechanisms involved in the renal tumor induction in the rat as well.

Haloacetonitriles have recently been identified as by-products of chlorination.[32] Data for four of these chemicals in the Salmonella/microsome assay are summarized in Table VI. As previously reported by[24] dichloroacetonitrile is positive in strains TA 1535, TA 98 and TA 100 in the absence of metabolic activation. The other three chemicals tested were inactive, both in the presence and absence of metabolic activation. However, it should be noted that the amount of dibromoacetonitrile that could be tested was severely limited by cytotoxicity.

Figure 3 compares preliminary results on the activity of the same four haloacetonitriles as tumor initiators in the mouse skin. The chemicals were administered in acetone topically to the shaved back of female SENCAR mice. This is followed by a promotion schedule involving the application of 1 μg of 12-0-tetradecanoyl-phorbol-13-acetate (TPA) 3 $\times$ weekly to the shaved back of these animals. At fifteen weeks the yield of papillomas was that shown in Figure 3. A clear dose-response relationship is observed for chloroacetonitrile and dibromoacetonitrile. It is too early to determine whether dichloroacetonitrile or trichloroacetonitrile are positive or negative in this assay. The results obtained with chloroacetonitrile and dibromoacetonitrile are expected to be reflected in the numbers of squameous cell carcinomas observed at one year based upon the

TABLE VI

Mutagenicity of haloacetonitriles in *salmonella* testor strains[a] with (+S9) and without (−S9) metabolic activation[b]

Chemical + Highest dose tested	TA 1535		TA 1537		TA 1538		TA 98		TA 100	
	−S9	+S9	−S9	+S9	−S9	+S9	−S9	+S9	−S9	+S9
Chloroaceto-nitrile 10 10 μl	—	—	—	—	—	—	—	—	—	—
Dichloroaceto-nitrile 1.5 μl	+[c]	+[d]	—	—	—	—	+	+[d]	+	+[d]
Trichloroaceto-nitrile 1.2 μl	—	—	—	—	—	—	—	—	—	—
Dibromoaceto-nitrile 0.05 μl	—	—	—	—	—	—	—	—	—	—

[a]Standard plate assay as per Ames *et al.* (1975).

[b]Data from Bull (1982).

[c]A positive response is defined as a minimum of twice the number of observed revertants/plates and a clear dose response relationship. A minimum of 6 doses was used for each compound.

[d]Revertants/plate were not significantly altered by the addition of S9 mix.

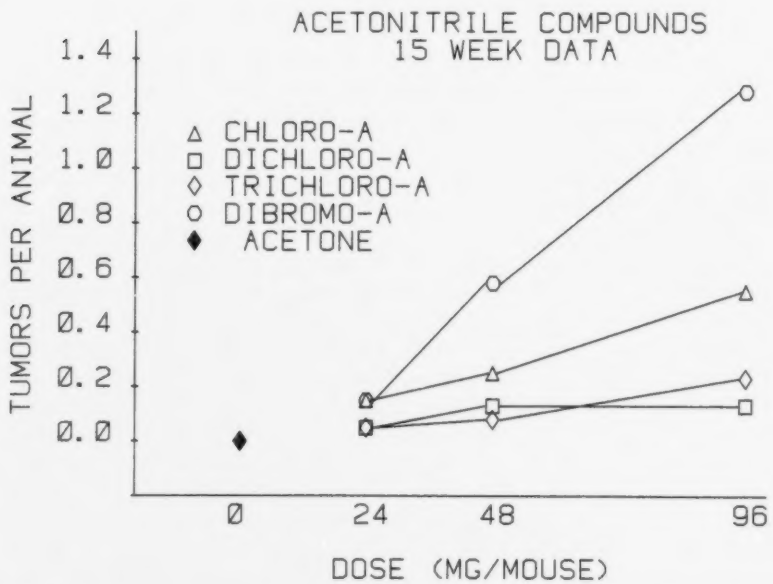

FIGURE 3 Tumor initiating activity of haloacetonitriles in the mouse skin. Animals were initiated with the total dose indicated divided into 6 applications in 0.2 and acetone. TPA was applied at a dose of 1.0 μg 3 × weekly beginning two weeks following the last initiating dose. Each point represents 40 female SENCAR mice.

observation of other workers.[33] Thus, it is already clear from these results that at least some of the haloacetonitriles are tumor initiators and will have to be treated as such in estimating the risks they pose to human health.

Summary and conclusions

The fact that chemicals can produce cancer by more than one mechanism is becoming clear. Although there is no specific data relevant to the nature of dose-response relationships at low response rates for any of these mechanisms, rationalizations based upon the somatic mutation theory of chemical carcinogenesis have been widely used. These rationalizations hold only for those mechanisms involving genotoxicity and are associated with tumor initiation. Basically, a rational argument can be made for irreversibility of such effects and a nearly absolute accumulation of damage. Such an argument does not necessarily hold for alternate mechanisms of chemical carcinogenesis. Such mechanisms would include cocarcinogenesis and promotion which do not lead to cumulative effects in the absence of initiated cells.

Epidemiological and lifetime feeding studies in experimental animals cannot distinguish between these mechanisms under normal circumstances. In either case it is virtually impossible to deal with pre-existing genetic, environmental and dietary factors that interact with the test chemical to produce a carcinogenic response. Such effects can even be the basis for disagreement between animal and human studies both in terms of the congruency of response to the same chemicals and their target organs.

Therefore, there is a genuine need to develop methods for assessing the mechanisms involved in producing cancer. This need is perhaps most clear today in differentiating tumor initiation from tumor promotion. There are a few bioassays which have been developed for this purpose. The mouse skin initiation/promotion system;[34] the rat liver foci system,[31] the bladder[16] and the colon[18] have been most thoroughly studied. However, even in these cases there is a need to examine the dynamics of the dose-response relationship at low response rates, particularly for promoters. Research in this area can have an important practical impact on regulatory policy as well as being of fundamental interest in understanding the processes involved in chemical carcinogenesis.

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